



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: )  
: Examiner P.K. Sripada  
HARALD BREIVIK, et al. )  
: Group Art Unit: 1202  
Serial No.: 07/902,500 )  
: Attorney Docket No.  
Filed: June 23, 1992 ) 1526.100 Cont. I  
:  
For: FATTY ACID COMPOSITION )

The Honorable Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

RULE 132 DECLARATION OF HARALD BREIVIK

I, Harald Breivik, a resident of Skjelsvik, Norway,  
do hereby declare as follows:

1) Qualifications: I am one of the applicants in the above-identified patent application (hereinafter "this application"). I hold a Ph. D. degree in Organic Chemistry, obtained in 1980, from the University of Oslo. From 1980 until the present I have been employed by Norsk Hydro a.s., assignee of this application, as a Senior Scientist in its Research Center in Porsgrunn, Norway. I currently head the Omega-3 Fatty Acids Group at the Research Center. Attached Exhibit A is a copy of my curriculum vitae, which accurately reports my scientific education, training, and experience.

2) Procedure and Results: In response to the rejection of this application, on grounds of unpatentability over the disclosure in U.S. Patent No. 5,130,611 to Cornieri et al., I have attempted to reproduce those specific Examples

in Cornieri et al. that disclose the preparation of concentrated mixtures of ethyl esters of  $C_{20+}$  fatty acids that contain a combined concentration of 80% or more of EPA+DHA. To do this, I and my colleagues followed as faithfully as possible the sequence of procedures described in Examples 2, 3, and 6-8 of the patent. Our objectives were (1) to ascertain whether those examples are operable to produce such a highly concentrated mixture of EPA+DHA and (2) if they are so operable, to ascertain what the EPA:DHA ratio is likely to be in the 80%+ resultant mixture, since that ratio is not disclosed in the examples themselves. The details of our work, and the results, are as follows:

#### Example 2 Replication

For practical reasons, we worked with a batch size that was half that reported in Example 2 of the patent, starting with 40 kg of fish oil, rather than 80. To a mixture of the fish oil and 25 kg of ethanol, in a closed reactor, was added 1.25 kg of concentrated sulfuric acid. The mixture was refluxed under nitrogen for six hours at a temperature of approximately 82° C. At the end of the reaction, heating was discontinued and excess ethanol was removed by distillation. The residue was cooled to room temperature and added to 100 kg of water and 75 kg of cyclohexane. The mixture was stirred and the water discharged. Water washings were repeated until the discharge showed a neutral reaction. The cyclohexane was removed by vacuum distillation at a temperature that varied from 61 to 70° C. (It was unnecessary

to also dry with anhydrous sodium sulfate, as the distillation is azeotropic and the water comes over with the cyclohexane. The H<sub>2</sub>O content after the distillation was only 76 ppm.) The product residue comprising EPA and DHA ethyl esters, which had the following composition, was stored under nitrogen for subsequent molecular distillation:

<u>Fatty acid</u>	<u>GLC area% <sup>1/</sup></u>
C14:0	7.3
C16:0	18.2
C16:1 n-7	8.3
Phytanate	1.5
C16:3 n-4	1.1
C16:4 n-3	1.4
C18:0	4.1
C18:1 n-7	3.5
C18:1 n-9	9.9
C18:2 n-6	1.3
C18:3 n-6	0.5
C18:3 n-3	0.7
C18:4 n-3	1.9
C20:4 n-6	1.1
C20:4 n-3	1.1
C20:5 n-3	14.6 EPA
C22:5 n-3	2.6
C22:6 n-3	10.5 DHA

### Example 3 Replication

The product of Example 2 was subjected to molecular distillation under a starting pressure of 10<sup>-3</sup> mbar (which is substantially the same as -- i.e., not significantly different from -- 10<sup>-3</sup> mm Hg<sup>\*\*/</sup>) and an evaporator temperature

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<sup>1/</sup> This is an approximation of weight percent, obtained relatively quickly by gas/liquid chromatographic analysis. Later in our work, as reported herein, actual weight percentages were determined for the most relevant products.

<sup>\*\*/</sup> 1 x 10<sup>-3</sup> mbar = 0.75 x 10<sup>-3</sup> mm Hg. This is as low as the pressure indicators go on Leybold KDL4 stills.

of about 108° C. (within the Cornieri et al. patent's range of "90-110° C") to remove natural and process impurities. (As would be expected, the pressure increased to 10<sup>-2</sup> mbar once the distillation was underway.) To guard against any aberrant results that might be caused by unseen equipment malfunction or idiosyncrasies, the product was divided into four parts, each of which was separately distilled, and two different molecular distillation stills were employed. Both were Leybold Model No. KDL4 stills: one located in Porsgrunn, Norway, at the Norsk Hydro a.s Research Center, the other in Sandefjord, Norway, at the facilities of Pronova Biocare a.s, a partially owned subsidiary of Norsk Hydro. The products obtained had the following compositions:

Residue Composition--First Distillation (90-110°C)

<u>Batch</u>	<u>EPA (GLC area%)</u>	<u>DHA (GLC area%)</u>	<u>EPA+DHA</u>	<u>EPA:DHA</u>
851-4* (Table 1)	20	17.4	37.5	1.15:1
851-27 * (Tables 2 and 3)	19.9	17.1	37	1:16:1
Res. 1** (Table 4)	20	17.2	37.2	1:16:1
Res:1** (Table 5)	18.0	15.7	33.7	1.15:1

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\* Distilled at Porsgrunn.  
 \*\* Distilled at Sandefjord.

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### Example 6 Replication

(As reported in the patent, Cornieri's Examples 4 and 5 led to concentrations of only 40-60% EPA+DHA, so we did not attempt to repeat them. Again, our objective was to achieve the 80%+ product reported in Example 8 of Cornieri et al. The patentees report that that was derived from the product of Example 7, which was in turn derived from the product of Example 6, which was prepared using the procedure of Example 5, but employing a higher evaporator temperature. Accordingly, we proceeded from Cornieri's Example 3 to his Example 6, as follows.)

Four samples of the products of Example 3 were subjected to molecular distillation, under the same starting pressure as in Example 3, but at temperatures in the range of 70°-80° C. The products had the following compositions:

#### Residue Composition--Second Distillation (70-80°C)

<u>Batch</u>	<u>EPA (GLC area%)</u>	<u>DHA (GLC area%)</u>	<u>EPA+DHA</u>	<u>EPA:DHA</u>
851-29* (Table 2)	26	26.1	52.1	1:1
Res:2.1** (Table 5)	21.2	18.8	40.0	1.13:1
Res:2.2** (Table 5)	23.1	27.6	50.7	0.84:1
Res:2.3** Table 5)	22.9	26.7	49.6	0.86:1

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\* Distilled at Porsgrunn.

\*\* Distilled at Sandefjord.

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As seen from the above results, Example 6 as replicated did not yield the 60-70% combined EPA+DHA ethyl ester concentration reported in the patent. It yielded only about a 40-52 % concentration, and that was as measured by GLC area, which tends for fish oil fatty acid mixtures to read somewhat higher than actual (or weight) percentages, especially if the composition has been repeatedly subjected to molecular distillation. This is because at distillation temperatures the polyunsaturated molecules tend to react together, forming oligomers. The high molecular weight oligomers tend not to pass through the chromatographic column, and thus are not included in the machine's calculation of the total sample size. The EPA and DHA, which do pass through the column, appear to the machine, therefore, to constitute a higher percentage of the sample than is actually the case. Nevertheless, the GLC area % analysis is useful because it can be performed quickly.

We repeated the distillation many times, altering pressures, temperatures, and feed rates, in an effort to maximize the EPA+DHA concentration. The highest that could be achieved by molecular distillation alone was 48.7%, on a weight basis. (See Batch No. 851-31 in Table 2, attached.) After several distillations of any one mixture, the EPA+DHA concentration was seen to decline, rather than increase. This is as expected, since the EPA and DHA molecules, being polyunsaturated, tend, as mentioned above, to combine to produce oligomers, the longer they are subjected to elevated

temperatures. Also, the EPA:DHA ratio became smaller and smaller, which also is as expected, since EPA is more volatile than DHA and, therefore, is preferentially removed in the distillate.

The results of all of the various distillations are reported in Tables 1-5, attached hereto.

#### Notes to Tables 1-5

The "A%" figures are area percentages as measured by gas/liquid chromatography. The "W%" is weight percent as determined by the analytical procedure described in Tande et al., *Journal of the American Oil Chemists Society*, 69: 1124 (1992). Because of the time involved, only selected products (as reported) were subjected to the weight percent analysis. After repeated distillations, the A% readings become less representative of the weight percent of EPA and DHA in the mixture. This is because a substantial amount of oligomers formed from the repeated heating of the mixture is retained on the GLC column, as explained above, and the A% readings are based solely on what passes through the column.

"Res." is the residue fraction, or "bottoms", from the distillation. "Dist." is the distillate fraction.

In addition to EPA and DHA, the products were also analyzed for DPA ( $C_{22:5n-3}$ ), because the concentration of the latter is known to build up significantly, in parallel with the DHA, as such mixtures are subjected to repeated distillation steps to remove the more volatile  $C_{16-18}$  fatty acids.



In each step, the reported EPA, DHA, and DPA contents were in the residue fractions. Step 2 involved the molecular distillation of the residue from Step 1. Step 3 involved the distillation of the residue from Step 2, and so forth.

#### Example 8 Replication

(Cornieri's Example 7 calls for the redistillation of the product of Example 6, but at a higher temperature. That essentially is what was attempted with the repeated distillations reported above. Having reached the maximum EPA+DHA concentration achievable through molecular distillation, we then subjected that product to Cornieri's final step in the concentration process, Example 8, as follows.)

The three highest-concentration products from Example 6 were selected for further treatment according to Example 8. Each product was added to a solution of urea in ethanol, and the mixture was shaken while heating under nitrogen. The ratio of ingredients was 1 g. of urea, to 6 ml. of ethanol, to 0.75 ml. of product (the same as 20 kg of urea in 120 liters of ethanol with 15 liters of product). After cooling, the resultant precipitate was separated and the remaining solution was vacuum concentrated to a small volume. After washing with water to remove all traces of urea, drying the organic solution, and removing the solvent by vacuum distillation, the product was subjected to molecular distillation as in Example 3, but at an evaporator

temperature in the range of 70-90° C. The composition of the resultant products, before and after the final molecular distillations, were as follows (also described in Tables 2, 3, and 4). These are true weight amounts, not the GLC area percentages:

Composition -- After Urea Fractionation and Final Molecular Distillation					
<u>Batch</u>	<u>Stage</u>	<u>EPA (Wt.%)</u>	<u>DHA (Wt.%)</u>	<u>EPA+DHA</u>	<u>EPA:DHA</u>
851-39 I (Table 2)	Pre-Distillation	10.8	43	53.8	0.25:1
851-39 II* (Table 2)	Post-Distillation	9.7	43	52.7	0.23:1
851-46 I (Table 3)	Pre-Distillation	13.7	41.4	55.1	0.33:1
851-46 II* (Table 3)	Post-Distillation	12.2	41.9	54.1	0.29:1
851-41 I (Table 4)	Pre-Distillation	12.2	40.9	53.1	0.3:1
851-41 II* (Table 4)	Post-Distillation	10.8	41.2	52	0.26:1

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\* Distilled at Porsgrunn.

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As seen from the above results, Example 8 as replicated did not yield the 80-90% combined EPA+DHA ethyl ester concentration reported in the patent. It yielded only about a 52-55% concentration (true weight percent).

3) Conclusions: The foregoing results demonstrate that the procedure disclosed in Examples 2, 3, and 6-8 of Cornieri et al. for extracting polyunsaturated fatty acid esters from fish oils is operable to prepare concentrates containing up to about 55 weight percent EPA+DHA, but not the "at least 80% by weight" concentration that we are claiming in our application. The results also demonstrate that the most concentrated EPA+DHA mixtures obtained by the Cornieri et al. procedure have EPA:DHA weight ratios in the range of about 0.25:1 to 0.33:1, and not within the range of "1:2 to 2:1" that we are claiming in our application.

4) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed by me to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent that might issue on the above-identified application.

Porsgrunn, Norway

Date: \_\_\_\_\_

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Harald Breivik

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3) Conclusions: The foregoing results demonstrate that the procedure disclosed in Examples 2, 3, and 4-6 of Cornieri et al. for extracting polyunsaturated fatty acid esters from fish oils is operable to prepare concentrates containing up to about 55 weight percent EPA+DHA, but not the "at least 80% by weight" that we are claiming in our application. The results also demonstrate that the most concentrated EPA+DHA mixtures obtained by the Cornieri et al. procedure have EPA:DHA weight ratios in the range of about 0.25:1 to 0.33:1, and not within the range of "1:2 to 2:1" that we are claiming in our application.

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Porsgrunn, Norway

Date:

1994-08-12

  
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